

MEASUREMENT OF GRAPEVINE CANOPY LEAF AREA BY USING AN ULTRASONIC-BASED METHOD

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Abstract

Aim: Measurement of leaf area in grapevine has always been a critical point in researches focused on irrigation management, training systems, source-sink interrelationships and efficiency of spray application to canopies. In this work, we propose the use of ultrasonic sensors as a fast and accurate tool for the estimation of large portions of leaf canopy area.

Methods and results: Through outputs of ultrasonic sensors installed on a tractor moving along vineyard rows, we calculated an ultrasonic-based leaf density index that we correlated with three measurements or estimates of canopy area: I) direct measurement of the area of a canopy portion (LAØ), assessed by summing the areas of all the leaves, where each single-leaf area was assessed by regressing the leaf diameter (the maximum width perpendicular to the main rip) against the related leaf area calculated on the basis of a relation between the leaf diameter and the leaf area, previously assessed through an area meter on a 20-leaf sample; II) the point quadrat output (LApq); and III) the canopy leaf area index (LAI) obtained through LAI-2000 (Li-Cor) technology. The measurements were assessed on six cultivars in three replicate rows (8-12 plants per cultivar per row) in a vineyard trained to a vertical trellis system.

Conclusion: When we correlated the three independent control parameters with each other, we obtained highly significant correlations between LApq and LAØ, but less significant correlations between these two and LAI-2000 outputs. Also, the correlations between ultrasonic outputs and LAØ and LApq were significant, with R^2 ranging between 0.84 and 0.85. On the contrary, no significant correlation was found between ultrasonic outputs and LAI-2000 outputs. These results were obtained by averaging all the values belonging to each replicated cultivar (10.5 m along the row, i.e., twelve contiguous vines); on the contrary, when the analysis was done over a shorter distance (3.5 m, i.e., four contiguous vines), the reliability of the ultrasonic-based method decreased.

Significance and impact of the study: These results point to the ultrasonic technology as a powerful tool to estimate large-scale leaf canopy area, with potential applications in precision farming. At the moment, however, the limitation of this approach is the requirement of reference values for leaf area (e.g., assessed by point quadrat) to obtain absolute and not only relative outputs. With this application we can quantify, in a few hours, the canopy of a whole vineyard, in order to analyze different vegetation zones or to follow canopy development.

Key words: canopy surface, canopy density, leaf area index (LAI), ultrasonic sensor, *Vitis vinifera* L., vertical trellis system

Résumé

Objectif : La mesure de la surface foliaire de la vigne a toujours été un point critique de la recherche sur l'irrigation, les systèmes de conduite, les relations source-puits et l'efficacité de l'application des produits de traitement en viticulture. Dans ce travail nous proposons l'utilisation de capteurs à ultrasons comme outils rapides et précis pour l'estimation de la surface foliaire à grande échelle.

Méthodes et résultats : À l'aide de capteurs à ultrasons installés sur un tracteur en mouvement dans la vigne, on a calculé un index de densité foliaire que l'on a mis en relation avec trois autres mesures ou estimations de la surface foliaire : I) mesure directe de la surface foliaire d'une partie de la végétation de la vigne (LAØ); II) le *point quadrat* (LApq); et III) l'index de la surface foliaire (LAI) obtenu par la technologie LAI-2000 (Li-Cor). Les mesures ont été effectuées sur six variétés conduites en palissage vertical avec trois répétitions de 12 plantes pour chaque variété.

Conclusion : En corrélant les trois paramètres évalués entre eux, on a obtenu des corrélations hautement significatives entre LApq et LAØ ; les corrélations étaient moins significatives entre ces deux mesures et les résultats obtenus à l'aide du LAI-2000. De plus, les corrélations entre la méthode à ultrasons et LAØ et LApq étaient significatives (R^2 entre 0.84 et 0.85). Au contraire, on n'a pas observé de corrélations entre la méthode à ultrasons et les résultats du LAI-2000. Les résultats ont été obtenus en moyennant les valeurs de chaque mesure répliquée (10.5 m le long du rang, c'est-à-dire douze plantes contigües) ; lorsqu'on a analysé les données sur des distances inférieures (3.5 m, c'est-à-dire quatre plantes contigües) la fiabilité de la méthode à ultrasons diminuait.

Signification et impact de l'étude : Les résultats obtenus avec les ultrasons ont démontré qu'il s'agit d'une technologie puissante pour estimer la surface foliaire à haut débit, permettant des applications dans le cadre de la viticulture de précision. Pour le moment, une limitation de l'application de ce type de mesure réside dans la nécessité de disposer de valeurs de référence de la surface foliaire (par exemple mesurée par le *point quadrat*) afin d'obtenir des résultats absolus et pas seulement 'relatifs'. Avec cette application il est possible de quantifier, en quelques heures, la surface foliaire d'un vignoble entier, pour mettre en évidence différents niveaux de développement végétatif au sein d'un même vignoble au cours de la saison.

Mots clés : surface foliaire, densité de végétation, indice de surface foliaire (LAI), capteur à ultrasons, *Vitis vinifera* L., système de conduite

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INTRODUCTION

Ultrasonic sensors have been extensively studied in agriculture as a way to improve pesticide distribution in order to reduce environmental pollution and enhance the efficiency of distribution (Giles and Downey, 2005; Gil *et al.*, 2007; Balsari *et al.*, 2008; Llorens *et al.*, 2011) with a variable application rate. A number of correlations have been found between the response of the ultrasonic signal and canopy thickness (Balsari and Tamagnone, 1998; Tumbo *et al.*, 2002; Zamahn and Salyani, 2004; Escolà, 2010). This was estimated by measuring the distance between the target and several ultrasonic sensors mounted vertically, facing the canopy. The distance is calculated by measuring the time between the emission and the reception of the signal, after it is reflected back from the target. However, the main limitation of this technique is that the accuracy of the response decreases as the target distance increases and also because of possible interferences between consecutive sensors (Escolà, 2010).

In this paper, the response of an ultrasonic sensor was used to calculate the distance from the target and obtain a leaf density value correlated to three independent measurements of leaf area, leaf density and leaf area index (LAI). The validation of a leaf density value (surface of the whole canopy related to units of row length) could allow the replacement of leaf volume (assumed to be estimated as the perfect parallelepiped describing the shape of the canopy) as a fundamental parameter in the development of applications for pesticide distribution. Moreover, a possible new use of ultrasonic sensors as a scientific instrument for large-scale leaf area measurement is suggested, particularly for a vertical trellis system. Indeed, leaf area is a fundamental parameter of vine physiology and is useful for the analysis of water and nutrient consumption, total photosynthesis and transpiration, yield and quality potential, light interception, and crop growth. The application of ultrasonic sensors being accurate, non-destructive, rapid and relatively inexpensive could putatively solve the main problems related to the measurement of leaf area (Pandey and Singh, 2011).

MATERIALS AND METHODS

1. Experimental field

This study was carried out in 2011 on three 61-m long rows of a vineyard planted in 2008 in Grugliasco (Turin, Italy), with a spacing of 2,5 m between rows and 0.9 m between plants, resulting in a density of about 4400 vines/hectare. The rows were made up of six cultivars (Barbera, Nebbiolo, Cabernet-Sauvignon,

Pinot Noir, Grenache and Syrah), for a total of 68 plants/row, with a post every four plants. Each row was divided into six randomly arranged boxes, five boxes containing 12 plants and one containing 8 plants, where each box contained plants of the same cultivar (Figure 1a). The vineyard was one-cane pruned (Guyot) to 12 buds per vine in a vertical trellis system. In all rows, Grenache plants were one year old and consequently poorly developed.

The measurements were made between the 81st and the 83rd growth stages of the BBCH scale (Lorenz *et al.*, 1994).

2. Canopy area measurement

Two types of techniques were used to measure leaf area or leaf density of the canopy: a direct measurement by counting leaf diameters (LAØ) (Manivel and Weaver, 1974) was applied to row number 2 and an inclined point quadrat (90°) (LAPq) method according to Wilson (1963) was applied to all rows.

The first method developed in two steps. The first step was to obtain, for each cultivar, an equation describing the relationship between the actual area measured by a portable area meter (Li-Cor LI3000) and the area of the square calculated on the maximum width (diameter = Ø) of a 20-leaf sample. The second step was to measure the diameters of all leaves in the 0.4-m high vegetative strip (between 1.05 and 1.45 m above ground) for each cultivar. The equation was applied to convert diameter to area. The sum of the areas represents the leaf area of the analyzed strip.

The second technique used was a close random point quadrat measurement (0.2-m interval), also performed in the 0.4-m vegetative strip between 1.05 and 1.45 m above ground. This simple technique consists in inserting a rod perpendicularly to the leaf canopy and counting the number of leaves touching the rod. The average number of foliar contacts corresponds to the number of leaf layers measured. Leaf area (m²/m) is then obtained by multiplying the number of foliar layers by the height of the strip measured (i. e., 0.4 m).

3. LAI estimation

LAI indirect estimation was made using an optical instrument, the LAI-2000 Plant Canopy Analyzer (Li-Cor, 1992). The instrument calculates LAI from observations made with a “fish eye” optical sensor, as described by Welles and Norman (1991) and Li-Cor (1992), which simultaneously measures diffuse radiation in five distinct angular bands around the zenith.

A row-crop canopy protocol, recommended in the instrument manual, was used (Li-Cor, 1992). One ambient light measurement above the canopy and four below the canopy were made. This procedure was repeated three times to estimate one LAI measure. A physical cap was used to limit the azimuthal field-of-view to 45°, facing away from the operator (Johnson and Pierce, 2004).

Measurements were made with low light at 8.00 a.m. It was not possible to assess LAI on Grenache because of the extreme amount of direct light that reached the instrument due to the small size of the young plants.

4. Ultrasonic technique

The ultrasonic sensors used in this research have been developed within the European project ISAFruit (Balsari *et al.*, 2008) with the objective to ensure precise, efficient, and safe spray applications in orchards, according to the actual needs of the crop in order to reduce the environmental impact of spraying.

Output from the ultrasonic sensors provides two types of information: i) the distance between the sensor and the target (Figure 1b), which indicates whether the target is present in front of the sensor and provides information about the calculation of the thickness of the canopy and ii) the density of the vegetation

(Figure 1c), mainly depending on the number of leaf layers and leaf orientation, which is calculated as an index of the ultrasonic echo signal. In fact, depending on the target density level, the analogical echo signal looks different and it is possible to get numeric thresholds corresponding to different levels of vegetation density. The density index is calculated as the integral of the echo signal as a function of time: a low value is the result of few echo signals received by the sensor following ultrasonic emission and means that the target in front of the sensor does not entirely intercept ultrasound emission (i. e., it is a rare canopy); on the contrary, a thick canopy results in a high value.

In this work the ultrasonic sensor has been installed on a vertical structure (parallel to the vineyard row at 1.25 m from the ground) connected to one side of a tractor moving at a speed of 1.5 m/s.

The sensor output is recorded as .txt file on a PC at intervals of 0.1 m. The results are a list of value: distance and density index. The data on leaf density is calculated on the basis of the echoes detected in time and can be influenced by factors external to the vineyard, therefore, for « holes » in the vegetation (distance = 0) the value of density was not considered in subsequent processing.

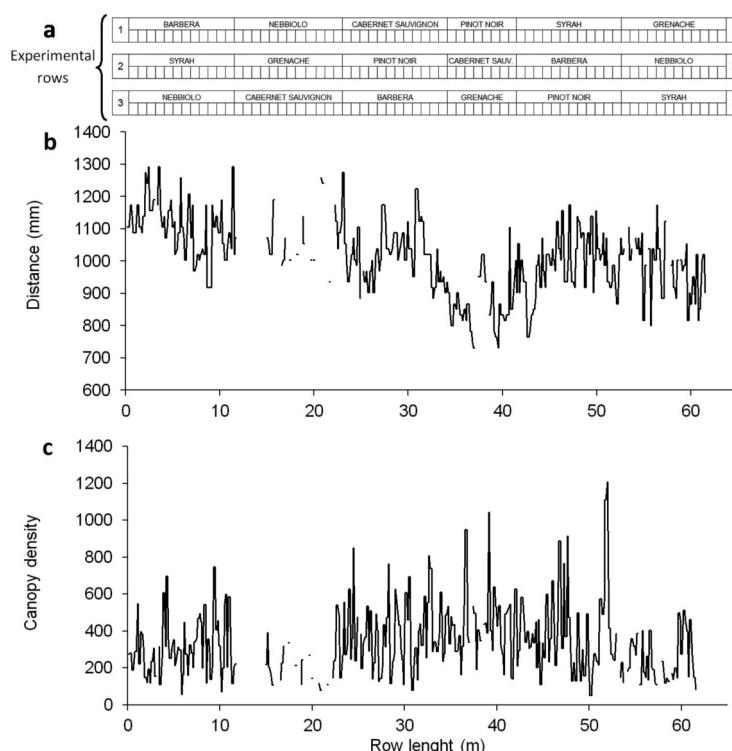


Figure 1 - Outputs of ultrasonic measurements after data cleaning. (a) Schematic representation of the three experimental rows: every little box represents a plant. (b) Distance between sensor and the canopy. (c) Canopy density relative to the 0.4-m high vegetative strip between 1.05 and 1.45 m above ground (exclusively row number 2, north side).

RESULTS AND DISCUSSION

Cultivar-dependent equations of the relationship between leaf area (y) (as obtained with the area meter) and leaf diameter (x) are shown in Table 1. A common equation could be used for all cultivar (Table 1). However, this common equation would result in an underestimation of leaf area of about 10 % for Grenache (which was quite different from the other varieties with respect to leaf morphology) and an overestimation of 1-5 % for the other cultivars. To avoid this problem, an equation for each cultivar was used in LAØ estimation.

Figure 2 shows the relationships among the three independent methods used to validate the ultrasonic technique, by examining the correlations between the leaf area of the canopy strip obtained by manually

measuring leaf diameters (LAØ) and LAPq and LAI. Each plot represents a whole cultivar in the vegetative strip of row number 2. The most significant correlation ($R^2 = 0.94$, Figure 2a and Table 2) was found between LAPq and LAØ, which suggests the point quadrat method can be considered as a reliable (and quite fast) method to be used as a standard reference. For the same purpose the relationship between LAI-2000 data and LAØ was assessed (Figure 2b). In this latter case, R^2 was lower than R^2 for LAPq and the regression line did not cross the origin of the axes. In terms of absolute values, a slight underestimation of leaf area can be observed with the point quadrat method compared to the direct LA measure (Figure 2a). This also occurred, albeit to a higher degree, with the LAI-2000 system, as reported by Patakas and Noitsakis (1999).

Table 1 - Cultivar-dependent equations of the relationship between leaf area (y) obtained by a portable area meter (Li-Cor LI3000) and leaf diameter (x) of a 20-leaf sample. An equation derived from data collected in all cultivars is displayed in the last row.

Cultivar	Equation	R^2
Barbera	$y = 0.6525 x^2$	0,97
Cabernet-Sauvignon	$y = 0.6981 x^2$	0,9
Grenache	$y = 0.7558 x^2$	0,94
Nebbiolo	$y = 0.6412 x^2$	0,91
Pinot Noir	$y = 0.6579 x^2$	0,97
Syrah	$y = 0.6937 x^2$	0,99
All cultivars	$y = 0.6809x^2$	0,96

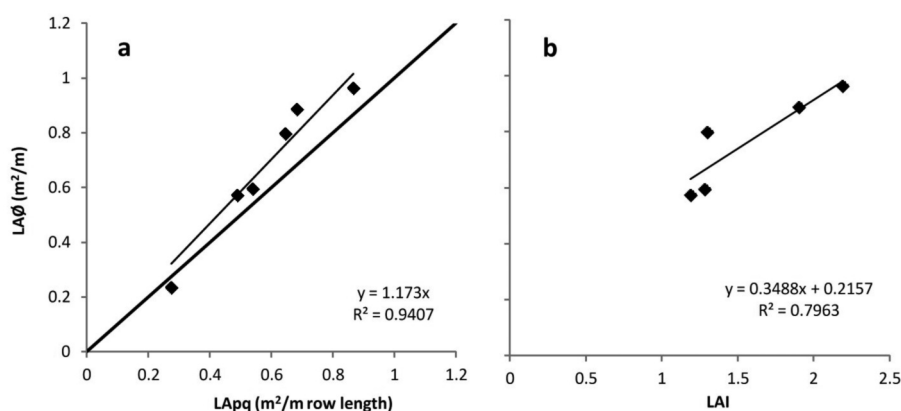


Figure 2 - Correlation between direct leaf area measurement (LAØ) and leaf area as measured by the point quadrat (LAPq) method (a) and leaf area index (LAI) as measured with LAI-2000 (b). The correlations relative to each of the six cultivars are shown (black boxes) except for Grenache in graph b. All data refer to the 0.4-m vegetative strip (between 1.05 and 1.45 m above ground) of plants belonging to row number 2, with the exception of LAI-2000 outputs.

Table 2 - Coefficients of correlation (R^2) among the various proposed methods for leaf area or LAI estimation obtained in row number 2. For the ultrasonic method, the mean data per cultivar obtained from the average of the two row sides were correlated. For R^2 relative to ultrasonic sensor *versus* LAPq and LAI-2000 see Figure 4a and c, row number 2. Asterisks mark significance of correlation (* $P<0.05$; ** $P<0.01$); n.s., not significant.

	Ultrasonic sensor	LAØ	LApq	LAI 2000
Ultrasonic sensor	1	0.84*	0.85*	0.57 n. s.
LAØ		1	0.94**	0.80 *
LApq			1	0.83 *
LAI 2000				1

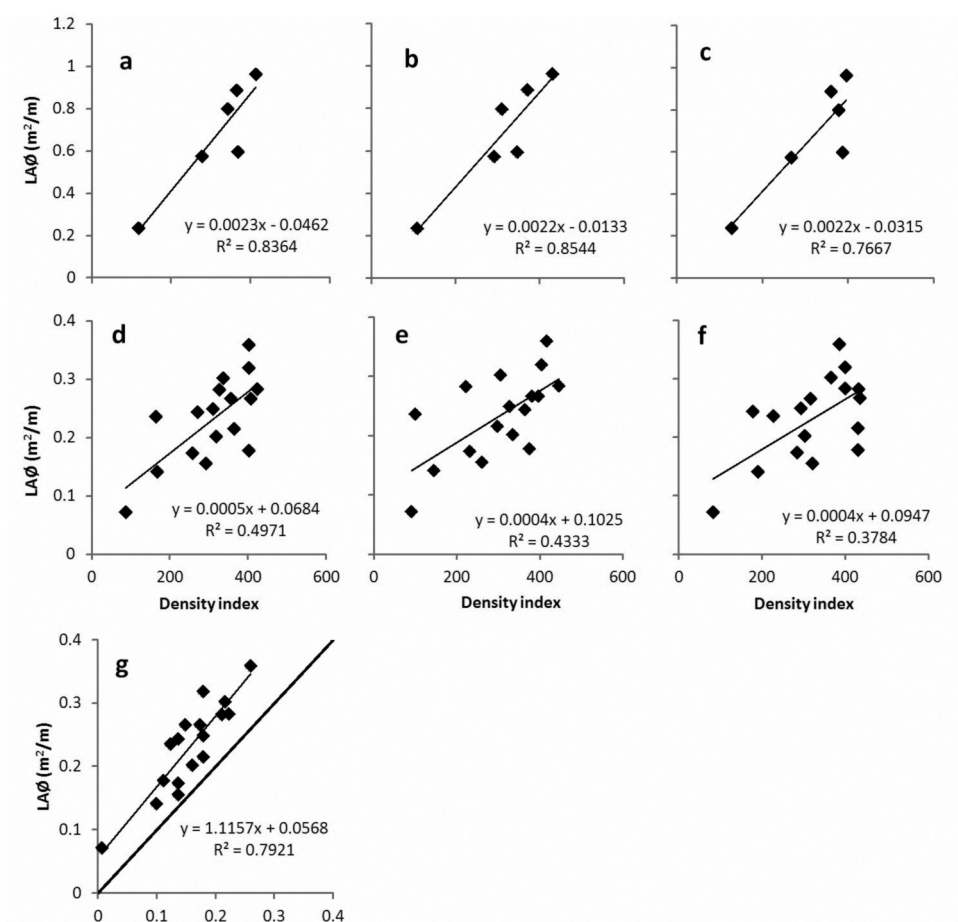


Figure 3 - Relationship between direct leaf area measurement (LAØ) and ultrasonic density index for each cultivar (a-c) (12 or 8 plants per cultivar) and every 4 plants (d-f). The graphs represent the average between the two sides of the row (a, d), the north side (b, e) and the south side (c, f). Graph g shows the correlation between LAØ and LAPq obtained from the average of four plants. All data were collected in row number 2 and refer to the 0.4-m vegetative strip (between 1.05 and 1.45 m above ground).

The ultrasonic density index was tested on row number 2, by comparison with LAØ outputs, on data obtained from the north side of the row, the south side, and the average of both sides (Figure 3), and by comparison with LAPq and LAI outputs on the whole experimental vineyard (i. e., rows 1, 2 and 3 ; Figure 4).

The density index was analyzed by grouping the data into cultivars (Figure 3a-c) or into groups of four consecutive plants (Figure 3d-f). The comparison of related R^2 allows to appreciate that the density index has a good reliability when averaged over a long distance along the row (about 10.5 m; Figure 3a-c), while reliability decreases if row length is shorter

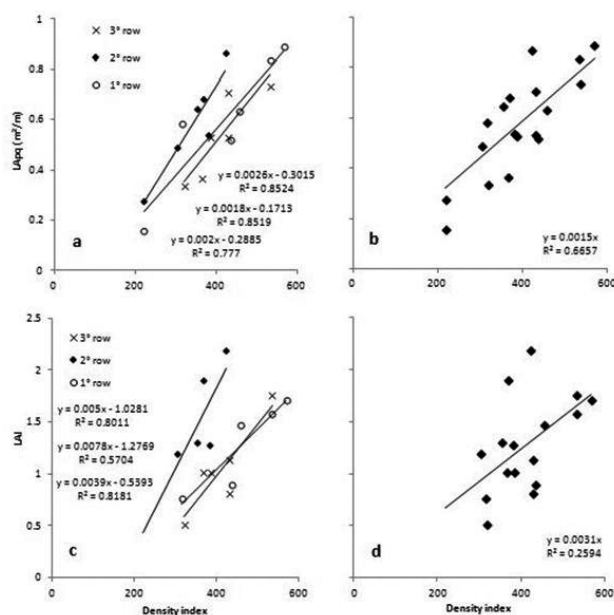


Figure 4 - Relationship between density index and leaf area as measured by point quadrat (LApq) (a, b) and leaf area index (LAI) as measured with LAI-2000 (c, d) for each cultivar in each single row (a, c) and from all assessed rows (b, d). All data refer to the 0.4-m canopy strips between 1.05 and 1.45 m above ground, with the exception of LAI-2000 outputs. Measures are divided according to cultivar and averaged from the two sides (north and south) of the row.

(3.5 m for each group of four plants). This decrease in reliability when smaller subsamples of vegetation are considered can also be observed in the correlation between LAPq and LAØ (Figure 3g). Similar results were discussed by Ollat *et al.* (1998), who showed an increase in reliability of LAI-2000 outputs when leaf area was measured from five plants in comparison with one single plant. The analysis of averages derived from the north, south and both sides of the row shows slightly higher R^2 values for the mean of the two sides (Figure 3a, d) compared to the north (Figure 3b, e) and the south (Figure 3c, f) sides. As no significant variation in R^2 was found when measurements were performed on the two opposite sides of the canopy compared with measurements performed on only one side, a repetition of the ultrasonic emission toward two opposite directions does not appear to be a valuable tool to increase the reliability of the measurement.

An asset in the estimation of leaf area with ultrasound is the ability to collect valid data even when the canopy is particularly poor, e.g., the data related to the young Grenache plants in our trial.

For the validation of the ultrasonic approach, the density index was compared with LAPq and LAI on three rows (Figure 4). When the comparison is made separately on all the cultivars from each of the three

rows, the results obtained show a high correlation to the point quadrat method, with coefficients of correlation ranging between 0.85 and 0.77 (Figure 4a). In the case of LAI-2000 measurements, the R^2 values are slightly lower ($0.57 < R^2 < 0.82$; Figure 4c), as reported by Llorenz *et al.*, (2011). Although there is a decrease in R^2 when all the data from the three rows are compared together (without being divided into rows), we obtain a satisfactory R^2 for LAPq *versus* ultrasonic index ($R^2 = 0.67$; Figure 4b). On the contrary, for LAI-2000 data the correlation is lost (Figure 4d). This problem prevents the formulation of a reliable equation employing LAI-2000 on which to base future studies.

CONCLUSION

During the past years several studies have reported the use of the ultrasonic method as a valid approach to estimate canopy presence-absence and thickness and thus to optimize pesticide distribution. Regarding canopy characterization interesting information was obtained on the correlation between ultrasonic and canopy width and volume. This work suggests the use of this type of sensor to estimate the leaf area of a vineyard for its advanced management, with other possible applications in canopy management such as thinning and control of leaf development. In addition, the use of this device as a tool to assess leaf area in

studies related to plant development could be considered for researches at the vineyard scale. The advantages of this method are the fast and easy data acquisition and processing that can be achieved with basic software. Moreover, with the application of GIS software in the field it would be possible to obtain a map showing areas with different vegetative developmental patterns within a vineyard in relation to various types of stress, similar to that obtainable with 'normalized difference vegetation index' (NDVI) sensor. Another strength of this method lies in the possibility of obtaining an accurate density estimation in poorly developed canopies, as it has been proved for the young plants of Grenache in this study. Finally, compared to optical sensors, ultrasonic sensors are not affected by light and could work in darkness.

However, the main limitations of this technique can be found in the low accuracy of the signal when the row length section analyzed is reduced and in the necessity of preliminary calibration (as point quadrat or LAI-2000 reference) in order to obtain a reliable absolute leaf area value. In addition, outputs of ultrasound devices do not allow to separate primary versus secondary leaf areas, an important information in vigour assessment (Trégoat *et al.*, 2001).

This study will need further development toward the measurement of canopies throughout their entire height by means of multiple ultrasonic sensors (a three-sensor combination has already been tested, data not shown) and toward an enhancement of the reliability of ultrasonic data in the case of short row length sections.

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